# STUDIES ON THE STRUCTURE AND PHYSIOLOGY OF THE FLIGHT MUSCLES OF BIRDS. 4. OBSERVATIONS ON THE FIBER ARCHITECTURE OF THE PECTORALIS MAJOR MUSCLE OF THE PIGEON

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Denny-Brown (1929), studying the red and white muscles of vertebrates, made some observations on the "light" and "dark" muscle fibers in the breast muscle of the pigeon. The later works on these two types of fibers have been reviewed by George and Naik (1957). More recently, George and Naik (1958a, 1958b) have shown that the red narrow fibers are rich in fat and mitochondria in sharp contrast to the white, broad, glycogen-loaded fibers, which contain only a negligible amount of fat and mitochondria. George and Scaria (1958a) histochemically demonstrated higher lipase activity in the red narrow fibers. The Krebs' cycle enzymes, too, seem to be localized in the narrow fibers (George and Scaria, 1958b). These findings have stimulated considerable interest and called for a basic understanding of the nature and disposition of the fiber components of this muscle as a whole. The present study, therefore, is an attempt to provide a comprehensive picture of the pattern of fiber distribution and the nature of the metabolite load in the different regions of the muscle.

## Materials and Methods

In order to obtain uniformly well developed *pectoralis major* muscle, only fully grown wild pigeons, either shot or trapped from a single locality, were used throughout for the present study.

### *Mapping the distribution of the two types of fibers in the muscle*

Due to the bipectinate arrangement of the fasciculi, it was found convenient to divide the muscle into twelve regions, each one extending to 10 mm. in length along a hypothetical line, drawn midway between the origin of the muscle fasciculi and the centrally placed tendon (as shown in Fig. 1). From each of these regions at the level of the aforesaid line, fresh frozen transverse sections were cut on a freezing microtome. Subsequently the sections were treated in the following manner. Transferring a fresh frozen section into distilled water or even saline or isotonic sucrose solution resulted in uneven curling up of the section. Again, the size of the muscle piece handled being large, some difficulties which were encountered in the beginning in obtaining a good entire section, were completely avoided by transferring the section directly into chilled 50% glycerol and mounting it on a microslide in the glycerol solution. In the preparations thus made the

arrangement of the fibers in the section, however large, was faithfully maintained with no distortions taking place. The glycerol-impregnated sections were thus found to be ideal to manipulate. Moreover, the sections left in glycerol solution and maintained at 0° C. can remain for more than a week without any perceptible defect and thus could be utilized for future observations.

The desired region of the mounted section was projected on the screen of a microphotographic camera at a magnification of 47 × and the photographic printing paper exposed directly to the image. "Normal" bromide papers were found

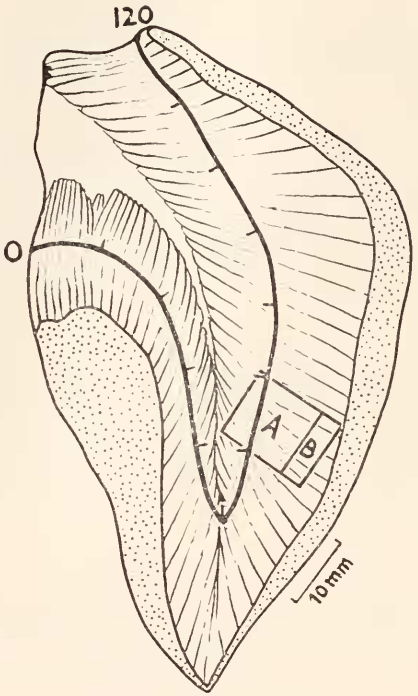


FIGURE 1. Dorsal view of the *pectoralis major* muscle of the pigeon showing the hypothetical lines 0–120 along which the distribution of broad fibers is recorded in Figure 2. The squares A and B indicate the regions of the muscle used for studying the variation in metabolite load and the structure at different depths of the muscle.

suitable. Using the sliding vernier on the stage of the microscope, continuous photographic records of the distribution of the broad fibers were made (Fig. 5). From such records by the method of random sampling, the mean value of the number of broad fibers per square mm. was determined for every mm. depth of the muscle. A survey of all the twelve regions was thus completed and a graph plotted illustrating the continuous distribution of broad fibers per square mm. at the distance of every 5 mm. along the line 0–120 (Fig. 1). The lines demarcating the areas containing 30–50, 50–70, 70–90, 90–100, 100–120 and 120–140 and 120–150 broad fibers per square mm. were drawn. The entire procedure was

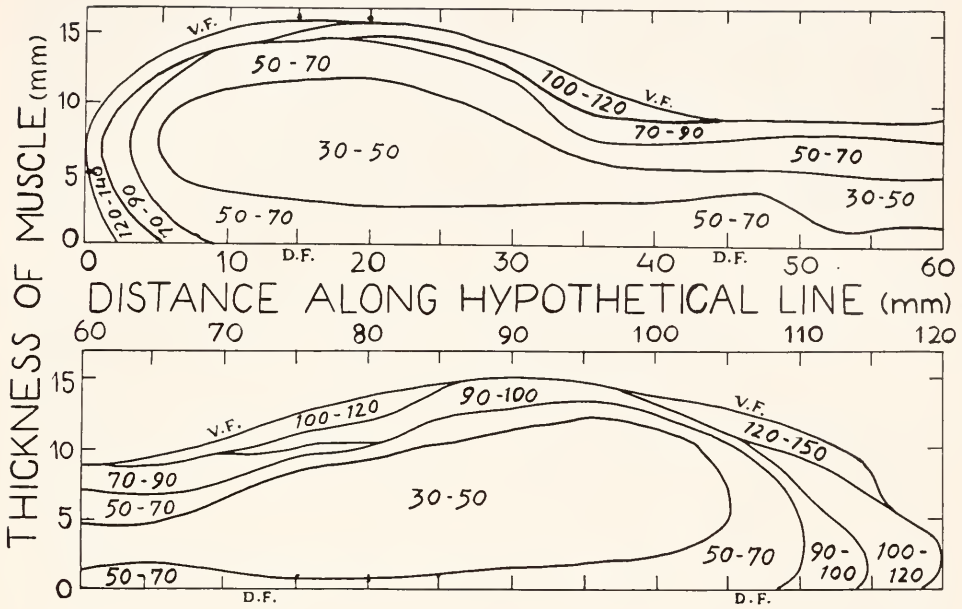


FIGURE 2. Cross-sectional view of the *pectoralis major* along the line 0–120 drawn in Figure 1. The figures in the chart show the number of broad fibers per square mm. D.F., dorsal face of the muscle; V.F., ventral face of the muscle.

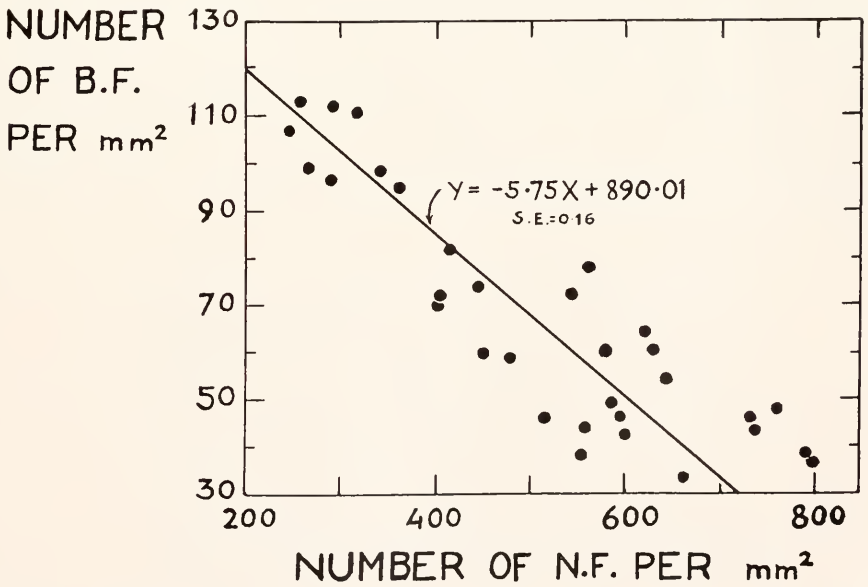


FIGURE 3. Relation between the number of broad fibers and the number of narrow fibers per square mm. of transverse section of the muscle.

repeated on the *pectoralis* of three pigeons. The results obtained are summarized in a graphical representation as shown in Figure 2. Since the individual variations in the *pectoralis* of different pigeons are considerable, the lines demarcating different areas in the figure are not claimed to be absolute, but they do show the

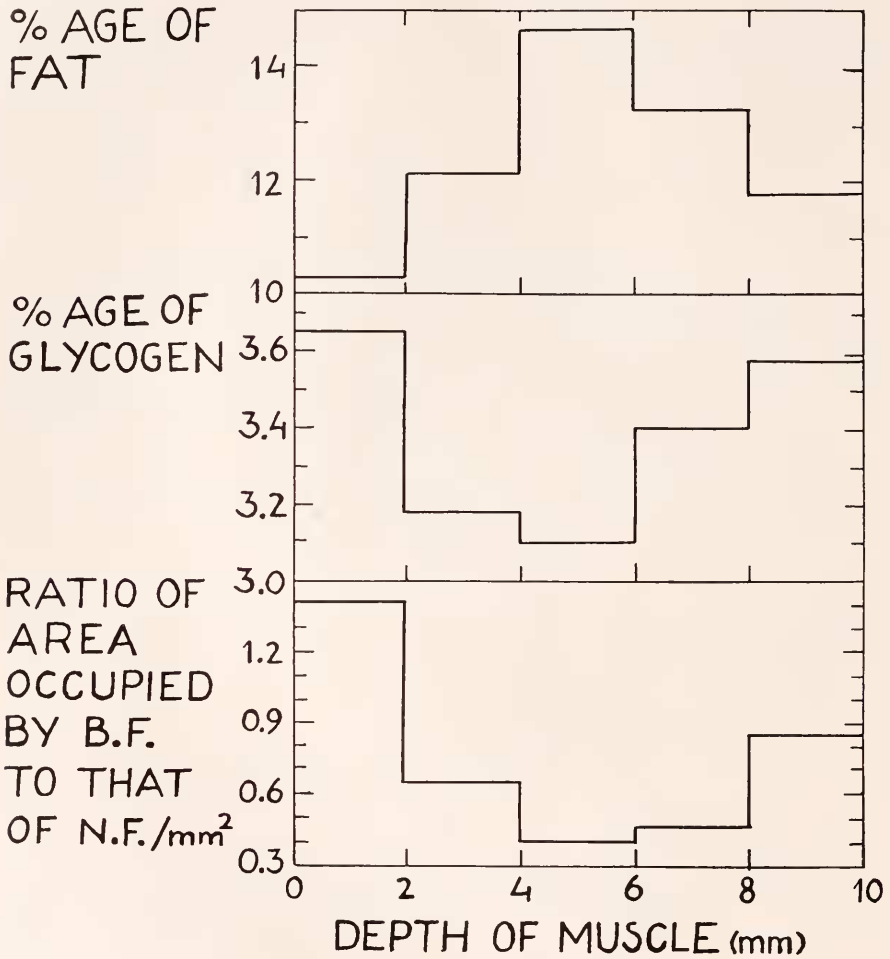


FIGURE 4. Variation in the percentage of glycogen and fat, in relation to the ratio of the area occupied by the broad fibers to that of the narrow fibers per square mm., at different depths of the muscle. The regions of the muscle marked A and B in Figure 1 were used.

generalized pattern of the distribution of the broad fibers in the *pectoralis major* muscle of the pigeon.

For counting the broad as well as the narrow fibers in one and the same region, the same procedure was adopted, except that the image of the section projected on the screen was magnified to about a hundred times, and the sections from the different typical regions of the muscle were used.

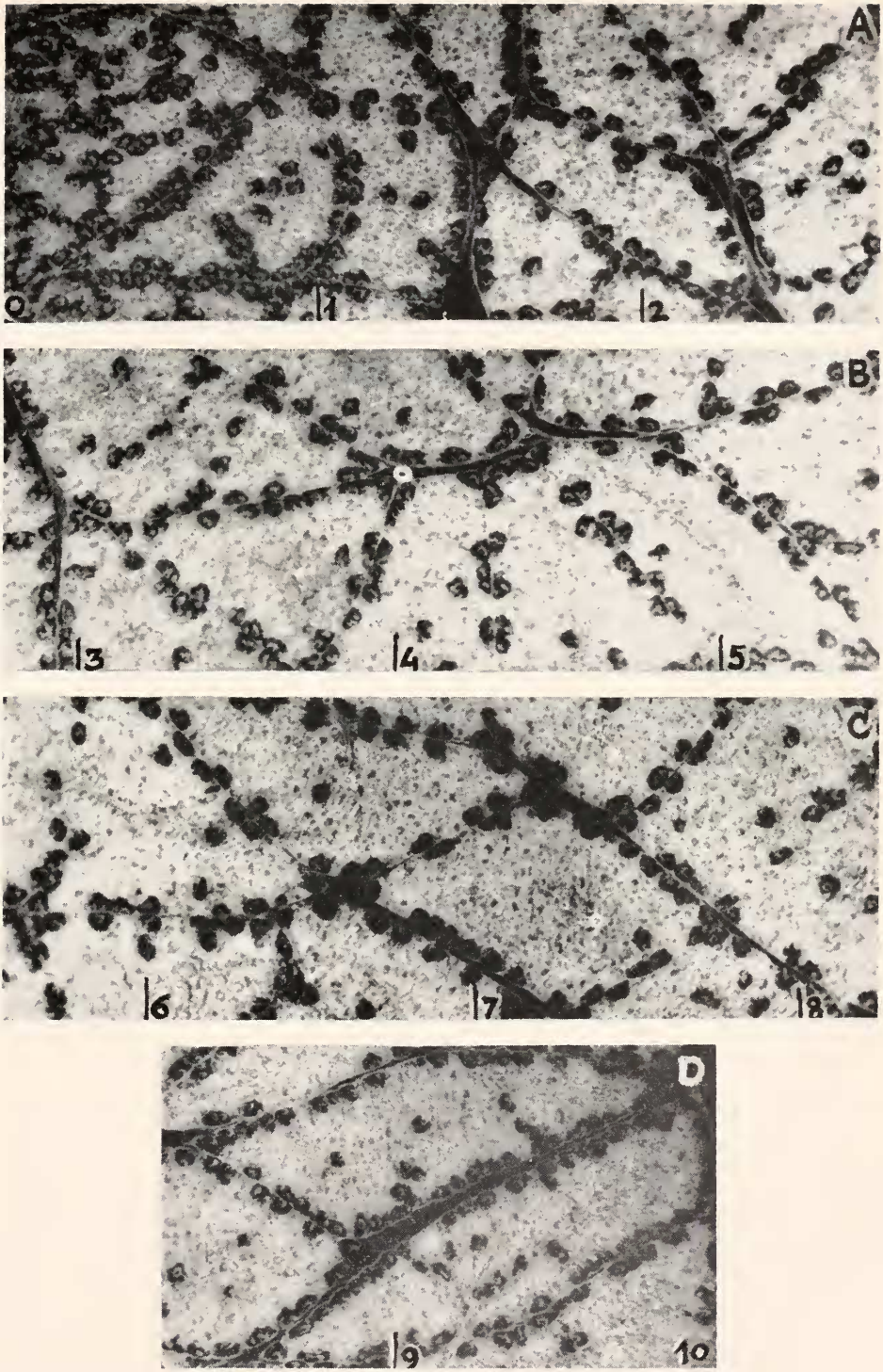


FIGURE 5. Negative prints of the transverse section taken from the region A (Fig. 1) showing the continuous distribution of broad fibers (darker in color) at different depths of the muscle. The numbers 1–10 on microphotographs indicate the depth in mm. from the ventral to the dorsal face of the muscle.

*Estimation of fat and glycogen at different depths of the muscle*

For the sake of convenience, the region of the muscle (marked A in Fig. 1) on the posteriormost end of the keel was used throughout. In this region the thickness of the muscle is only about 10 mm. and the variation in the distribution of the broad fibers at the different depths of the muscle is gradual. From this region A, a piece about 10 cubic mm. in size was cut out for the estimation of glycogen and a somewhat bigger piece for the estimation of fat. From a region B lateral to A, another piece was cut out and transferred to the freezing chamber of the refrigerator and used later on for studying the distribution of the broad fibers in this region by the method already described.

The muscle piece cut out from region A was mounted on the stage of a freezing microtome so as to obtain horizontal sections. It was frozen hard, the outermost epimysium was peeled off with a pointed forceps or sliced off by a superficial stroke of the microtome knife, and 1-mm. thick slices of the muscle were serially cut. Since all these horizontally cut sections were of uniform and known thickness, each could be said to represent the nature of the muscle tissue at a known depth. The thickness of the sections was not actually measured since the microtome used was a brand new "Sartorius" model and all the possible precautions, such as avoiding the fluctuations in the temperature, were taken so as to obtain sections of uniform and accurate thickness. Each frozen section was immediately transferred to a weighing bottle and dehydrated. The sections to be used for the estimation of glycogen were dehydrated in a vacuum-desiccator at one atmosphere pressure and maintained at 0° C., whereas for fat extraction, sections were dehydrated in an air-oven at 80° C., and finally in vacuum.

The dehydrated sections were weighed and their glycogen content was estimated according to the method of Kemp *et al.* (1954). For the quantity of the muscle used for estimation (about 20–30 mg. per dry weight) it was found necessary to dilute the glycogen extract in the deproteinizing solution to 10 ml. The color developed was measured on the Beckman spectrophotometer (DU model) at 520 $\mu$. For the estimation of fat the dehydrated material was ground and, after weighing, transferred to a fat-extraction thimble. The fat was extracted in the Soxhlet apparatus with 1:1 ethanol-ether mixture (George and Jyoti, 1955). About 70–100 mg. of dehydrated muscle were used for each estimation.

*The estimation of glycogen in the two types of fibers*

Small pieces from the breast muscle of a decapitated pigeon were cut out and dropped in previously chilled 80% methanol and left undisturbed at − 10° C. for 24 hours. The fibers from the muscle thus preserved were teased out in methanol under a binocular dissection microscope with watch-maker's forceps. The two types of fibers were isolated and transferred to two separate containers containing methanol and fitted with air-tight glass lids and stored in the refrigerator. Sufficient numbers of fibers which would yield about 2–5 mg. in dry weight were isolated and collected for each estimation. These fibers were then removed from the methanol solution, dehydrated in vacuum and weighed on a microbalance. Glycogen was estimated, as already mentioned, by the micromethod of Kemp *et al.* (1954).

## Results

Figure 5 presents a typical picture of the distribution of broad fibers in the muscle. In each fasciculus the broad fibers are mainly concentrated towards the periphery. This pattern is maintained throughout the muscle. In regions of the muscle where there are larger numbers of broad fibers or lesser numbers of narrow fibers, the fasciculi have a smaller cross-sectional area with broad fibers closely packed along their borders without any intervening narrow fibers. The number of broad fibers per square mm. in the different regions of the muscle is shown in Figure 2. The relation of the number of broad fibers to that of the narrow ones per square mm. is shown in Figure 3. From both these, the number of broad fibers, as well as the number of narrow fibers per square mm., in any region of the muscle could be approximately determined.

The variation in the metabolite load and the number of broad fibers per square mm. at different depths of the muscle are indicated in Table I, while in Figure 3 the same data are utilized to show the relation between the structure of the muscle

Table I

*The number of broad fibers per square mm. and the percentage of fat and glycogen at different depths of the breast muscle of the pigeon. (The portion of the muscle marked A in Fig. 1 was used. The figures indicate the average values of six sets of readings)*

| Depth of the muscle in mm. (starting from the ventral face) | Number of broad fibers per square mm. ±S.D. | Percentage per dry weight of the muscle ±S.D. | |
|---|---|---|---|
| | | Glycogen | Fat |
| 0–2 | 90 ± 14 | 3.655 ± 0.275 | 10.289 ± 1.942 |
| 2–4 | 63 ± 8 | 3.175 ± 0.054 | 12.095 ± 1.056 |
| 4–6 | 48 ± 3 | 3.102 ± 0.127 | 14.632 ± 1.752 |
| 6–8 | 51 ± 4 | 3.409 ± 0.184 | 13.250 ± 0.571 |
| 8–10 | 72 ± 9 | 3.588 ± 0.236 | 11.743 ± 0.572 |

and the metabolite load. The number of narrow fibers for the corresponding number of broad fibers was calculated by using the formula of the regression line in Figure 3 and the ratio of the area occupied by the broad fibers to that of the narrow fibers in square mm. was determined by using the mean value of the diameter of these fibers. The diameter of the broad fibers is 69.00 ± 14.00 $\mu$ (1000) and that of the narrow fibers is 30.11 ± 6.56 $\mu$ (2000). The figures given in parentheses indicate the number of fibers measured from the fresh frozen sections taken from the various regions of the muscle.

The values of the glycogen content of the broad and narrow fibers, calculated on the dry weight of the muscle preserved in methanol, are, respectively, 10.240 ± 0.093% and 2.464 ± 0.311% (each value is the mean of three readings). Methanol removes much of the fat (mainly from the narrow fibers) and some of the amino acids.

## Discussion

It has been known that in many active muscles, the muscle fibers towards the periphery become larger in diameter and lighter in color, compared to those in

the interior. In such muscles even in the individual fasciculus, the light fibers are situated towards the periphery. In the pigeon breast muscle, the white broad fibers and the red narrow fibers show a somewhat similar distribution pattern but these fibers differ from the light and dark fibers of the other muscles in that they are sharply differentiated into two distinct types without any intermediate forms. The broad fibers are glycogen-loaded and poor in fat inclusions and mitochondria, whereas the narrow fibers are fat-loaded and have a high mitochondrial content and are poor in glycogen (George and Naik, 1958a, 1958b).

In a single muscle uneven distribution of metabolites has been long since realized. To reduce such localized variation to the minimum, customarily a large piece of muscle is utilized for the estimation of metabolites. Present work shows that in a muscle like the *pectoralis major* of pigeon having heterogeneous cellular elements, variation in metabolites in the different regions of the same muscle and even in a single fasciculus is quite large. Needless to say, what applies to glycogen and fat might equally apply to other chemical constituents in which the two types of fibers differ.

A general belief that the muscle fibers towards the periphery of the muscle are more active than those in the interior and, due to higher activity, increase in diameter, does not seem to hold good, at least in the case of the *pectoralis* of pigeon. Undoubtedly, the red fibers of pigeon breast muscle, due to their remarkably well developed enzyme systems, play a major role in effecting the sustained contractions of the muscle. In white fibers, on the other hand, the oxidative processes are not developed or developed only to a negligible extent, in that the dehydrogenase activity in these fibers, as shown by histochemical method, is negligible or nil (George and Scaria, 1958b). All the same, the white fibers are not inactive elements of the pigeon breast muscle. In the normal animal they show no signs of atrophy. A glycerinated white fiber of pigeon breast muscle contracts in the same manner as a glycerinated red fiber of the same muscle on the addition of ATP. The study on the reactions of these two types of fibers to experimentally induced disuse atrophy has yielded significant results. When the movement of the humerus is restricted for three months by a plaster cast, the white fibers in the deeper layer of the muscle show acute sign of atrophy whereas the red fibers appear practically unaffected (George and Naik, unpublished data). These findings suggest the possibility of some differences in the mechanical properties of the two types of the fibers and in that case some physical factors may underlie the distribution pattern of the two types of fibers in the muscle.

Denny-Brown (1954), has shown that a single nerve in the breast muscle of pigeon can innervate both, the red as well as the white fibers. Since the activity of these muscle fibers must be conditioned by the fundamentally different chemical system in them, it is difficult to believe that the amount and the mode of activity performed by these two types of fibers are the same. In what exact manner the white fibers contribute to the activity of the muscle is far from clear and as a prelude to such an understanding, an extensive study of these fibers is essential. For such a study Figure 2 can be a useful guide. Moreover, the method used in the present work to study the variation in the metabolite load in relation to the variation in the fiber make-up of the muscle, can be used for studying the distribution of various constituents such as enzymes, amino acids and minerals in the muscle.

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## Summary

1. The relative distribution pattern of the red and white muscle fibers in the breast muscle of the pigeon is studied.

2. There exists a direct relation between the distribution of metabolites and that of the two types of fibers in the different regions of the muscle.

3. Quantitative estimation of glycogen in the two types of fibers confirms the higher concentration of glycogen in the white fibers.

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